In this case, the vaccine composition (Group I) and the method of using it (Group II) are directly connected and unified as a final product and its intended use. The vaccine composition of Claims 1-19 comprises an inactivated or killed *E. coli* O157:H7 bacterin in combination with a metabolizable oil and optionally a pharmaceutically acceptable carrier. This composition is ready to use for the effective immunization and safety in treating animals in the method of Claims 20-21 for reducing the shedding of *E. coli* O157:H7. Contrary to the Examiner's opinion, with all due respect, the inactivated or killed bacterin formulation could not be used for protein expression. Rather, protein expression in *E. coli* requires a live culture and cannot be achieved with an inactivated or killed bacterin.

Moreover, there is no statutory prohibition against claims drawn to both product and process of use residing in the same issued patent. In sum, the Office has failed to justify the restriction requirement. Applicants urge the Examiner to reconsider and withdraw the requirement to restrict this application.

Consistent with the foregoing remarks and in accord with the requirement of 37 C.F.R. § 1.143, Applicants confirm the provisional election that had been made with traverse to prosecute the invention of Group II, Claims 20-21, drawn to a method for reducing the shedding of *E. coli*.

Applicants currently retain the nonelected subject matter to afford the Examiner the opportunity to reconsider the restriction requirement and, thus, for future consideration on the merits. It is to be understood that the provisional election is for procedural purposes only and that Applicants reserve the right to file a divisional application directed to the nonelected subject matter of this invention in the event that the restriction requirement is upheld.

Applicants gratefully acknowledge that the Examiner has deemed that the information disclosure statement of January 10, 2005 was in compliance with the provisions of 37 C.F.R. § 1.97 and has considered the cited documents.

The Examiner comments that the use of the trademark Emulsigen in the application should be capitalized and accompanied by the generic terminology. To expedite matters towards an early allowance, the present amendment provides trademark recitations in conformity with the Examiner's guidelines.

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The Examiner objects to Claim 21 due to the misspelling of *neomycin* and *acidophilus*. In response, the present amendment corrects these obvious typographical or clerical errors.

The Examiner rejects Claims 20-21 under 35 U.S.C. § 112, first paragraph, for failing to comply with the enablement requirement for reasons set forth on pages 4-8 of the Office action. Basically, the Examiner believes that the specification does not provide substantive evidence that the claimed vaccines are capable of inducing protective immunity. The Examiner raises many questions about the working examples in the specification. Applicants respectfully traverse the rejection.

First of all, working examples are not even a statutory requirement for patentability. Simulated examples are acceptable. Yet, Applicants have provided working examples with good results: The safety of the vaccine in cattle is demonstrated in Example 2 and the efficacy of the vaccine in reducing pathogen prevalence is established in the field study of Example 3.

For the most part, Applicants' materials and methods in their exemplification of the invention are given in excellent detail. Granted, there are some omissions in certain minor points of the experimental data recited in the application. For instance, how *E. coli* was inactivated, what happened to the other groups of cattle, what was the standard vehicle used for comparison, why were some titers high before injection, what was the control in the field study, and the like, were not explained in text. However, the lack of these tiny, insignificant points in the experimental studies is not fatal to the patenting of the vaccine composition and the method of using it for the plain and simple reason that the omitted information is inconsequential to the description of the invention.

Without a doubt, Applicants have adequately described the present invention in the specification to enable the ordinary practitioner to practice the claimed invention without undue effort. Examples of the formulation ingredients, effective dosages, routes of administration, dosing regimens and the like have been fully disclosed in the application. The practitioner would have no reason to question the truth or accuracy of Applicants' assertions that the vaccine composition of the invention can be used to reduce the shedding of *E. coli* O157:H7. There is no reason to believe otherwise. Besides, it is well within the ordinary skill of the pharmaceutical and veterinary arts to be able to repeat the demonstrated safety and efficacy of the vaccine in cattle through routine effort.

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All of the missing information regarding the experimental data, questioned by the Examiner, is not critical or essential to the actual practice of the invention. For example, how to inactivate bacteria is a well-known, routine procedure for researchers that can be found in many publications. While Applicants followed the standard method in the pharmaceutical and veterinary industries by employing formalin, the particular procedure used to inactivate *E. coli* is not an important aspect of the present invention.

Similarly, what happened to the other groups of cattle is irrelevant to the disclosure of the claimed invention. To answer the Examiner's query, however, it has been determined that Groups 1-4 were apparently used for other studies of different vaccines being evaluated at the same time as the instant *E. coli* vaccine. It was an accidental oversight that the numbers of the groups and cattle did not get revised before the non-provisional application was filed. Nevertheless, and more importantly, it is confirmed for the record that all experiments performed with *E. coli* are fully disclosed in the present application.

With respect to the standard vehicle used for comparative purposes in Example 2, it has been found that Applicants used art-recognized aluminum hydroxide in the vaccine of Group 6 in order to show the improvement when the novel metabolizable SP oil is added to the aluminum hydroxide adjuvanted formulation (Group 7). Over 60 years ago, alum-adsorbed allergen extracts were first used for depot vaccination and since that time, aluminum hydroxide continues to be the most common adjuvant in vaccines. Although Applicants did not state the standard vehicle as such, the purpose of the comparison, *i.e.*, to demonstrate that the vaccine composition of Group 7 was effective and safe, was still satisfied. Group 7, having the greatest overall serological titers, showed the best improvements in immunity; and the animals displayed minimal, normal reactions at the vaccine administration sites.

In terms of the significant variation in a few titers, some abnormalities are to be expected under the circumstances of working with live animals. Certainly, Applicants utilized a good control facility and exercised care during the experiments. However, *E. coli* is ubiquitously found. The calves that had a high titer before injection (calf # 292) or had an increased titer without treatment (calf # 283) might have been infected with another *E. coli* strain and peaked as a cross-reaction despite the attempt to keep the controls clean of infection; or perhaps one of the calves had been exposed accidentally to *E. coli* O157:H7. Any

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practitioner who works in the veterinary field with cattle would appreciate these and other possible reasons for certain titers to be anomalous.

The more important value of the titers is seen in the overall average of each group of animals involved in the study, namely 1184 for those treated by the vaccine of the invention, compared to 868 for those animals treated by the conventionally adjuvanted vaccine and further to 735 for the controls. By and large, the controls stayed at the baseline titer of 640 (a normal range) and the titers of the conventionally adjuvanted vaccine remained the same over time. Yet, unexpectedly, the vaccine composition of the invention demonstrated a significant improvement in titers.

The reaction scores show that the vaccine of the invention is safe on administration. With all vaccinations, a little lump is expected when the active ingredient is released slowly from the site of depot administration. It is typical, though, for vaccines that give a higher immune response to cause a greater reaction. Because a severe lump usually forms from vaccines with significantly higher immunogenic responses, it was initially thought that the claimed vaccine composition would cause a greater adverse reaction. Surprisingly, the numbers were close on comparison and no major reaction had been observed. Despite the higher immune response, the results demonstrated that the size of the reaction lump was the same as the traditional vaccine and thus no safety issue was found.

Regarding the control group in Example 3, one would readily appreciate that the control group represented those animals that did not get vaccinated. The field study was what is called a "nature challenge" where there is no artificial challenge; the animals are just subjected to the pathogens encountered normally in the field. The study was performed and described in the application to show the extent of the reduction compared to non-vaccinated controls. The reduction of pathogens in the field by 20.3% on hide samples and 31.1% in fecal samples illustrate very good results in support of the claimed method.

To explain some of the standard terminology used in the application ("intervention strategies," "hide samples," *etc.*) for the benefit of the Examiner, a true copy of a study published online by the National Cattlemen's Beef Association is provided herewith. The study also demonstrates how well Applicants' vaccine might be used in actual practice with other interventions to reduce the shedding of *E. coli* O157:H7.

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In view of the foregoing comments, there is no doubt that the ordinary practitioner would understand the description of the present invention and be able to practice it without undue experimentation. Thus, it is respectfully requested that the Examiner withdraw the rejection of the claims under 35 U.S.C. § 112, first paragraph. It is further asked and respectfully urged that the Examiner reconsider holding that the present application may claim the full priority benefits of the provisional Application No. 60/454,182 for art rejections.

The Examiner rejects Claim 20 under 35 U.S.C. § 102(b) as being anticipated by Finlay *et al.* for reasons set forth on page 9 of the Office action. Applicants respectfully traverse the rejection for the following reasons.

In the Examiner's last sentence on page 9, it is stated: "[T]he burden is on applicant to show a novel or unobvious difference between the claimed product and the prior art." With all due respect, this is not the true standard for 35 U.S.C. § 102(b). Rather, the statute only requires novelty. Unobvious differences are only relevant to a rejection under 35 U.S.C. § 103, which is not being applied against Claim 20.

With respect to the rejection at hand, therefore, anticipation requires an identity of invention between Applicants' claimed invention and the cited reference. Every element of the claimed invention must be identically described in the cited reference of Finlay *et al.*

Looking at what Finlay *et al.* actually teach, the reference describes compositions that employ the cell culture supernatant derived from an *E. coli* culture. In the process of making the concentrated supernatant, the whole cells are removed by centrifugation [0107]. Finlay *et al.* only use whatever protein antigen is released in culture since the reference bases the effectiveness and virulence of the supernatant in use as a vaccine totally on the antigenic protein content - particularly those secreted by the type III system. Finlay *et al.* claim that the proteins are the major targets of the immune response in humans following infection and state that cattle do not usually mount a significant serological response against these proteins following natural exposure to the organism [0132]. In no uncertain terms, Finlay *et al.* do not describe the use of any bacterial vaccine, let alone Applicants' novel inactivated or killed whole or subunit *E. coli* bacterin as a vaccine for reducing the shedding of *E. coli* O157:H7.

Since the claimed invention is not identically disclosed by the reference, it is respectfully asked that the rejection of Claim 20 under 35 U.S.C. § 102(b) be withdrawn.

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The Examiner rejects Claim 21 under 35 U.S.C. § 103(a) as being unpatentable over Finlay *et al.* as applied to Claim 20 above and further in view of Brashears *et al.* for reasons set forth on pages 10-11 of the Office action. Applicants respectfully traverse the rejection for the following reasons.

To establish a *prima facie* case of obviousness, the guidelines of M.P.E.P. § 706.02(j) and case law provide three basic criteria: (1) There must be some suggestion or motivation to modify the reference or to combine the reference teachings; (2) there must be a reasonable expectation of success; and (3) the combined references must teach or suggest all claim limitations.

There is a serious health issue from *E. coli* O157:H7 infection of beef cattle. In fact, there is a weekly report published on the statistics of the morbidity and mortality caused by the *E. coli* O157:H7 bacterin. There is a definite art-recognized need for a vaccine that provides improved protection against *E. coli* O157:H7 infection and reduction in shedding of the *E. coli* O157:H7 bacterin. Surprisingly, the new vaccine composition of the present invention solves the long-standing problem by demonstrating significant immune responses and protection against *E. coli* O157:H7 infection.

Examining what the collective art fairly teaches to the ordinary practitioner, it is clear that the practitioner would not arrive at the claimed invention. The art fails to provide any suggestion or motivation of the desirability of combining the references and doing what the inventors have done. Even if combined, the practitioner would still find real distinction between the claimed invention and the cited references. It is clear that the combined references do not teach or suggest all of the critical elements of the claimed vaccine formulation and the method of using it.

As previously noted above, the primary reference of Finlay *et al.* only shows compositions that employ the cell culture supernatant derived from an *E. coli* culture. Activity of the art composition is based totally on the protein antigen that is released in culture. The reference teaches that cattle do not usually mount a significant serological response against the antigenic proteins following natural exposure to the organism. There is no disclosure or suggestion of the value of using the bacteria itself in a vaccine. Finlay *et al.* actually discard the whole cells because they do not appreciate any benefit of using the whole bacterial cell.

In sharp contrast and quite unexpectedly, Applicants were successful in significantly reducing the shedding of *E. coli* O157:H7 through treatment with a whole cell vaccine. Thus,

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if anything, Finlay *et al.* teach directly away from Applicants' method of using a vaccine composition that contains the inactivated or killed whole or subunit *E. coli* bacterin. Based on the teachings in the primary reference, the ordinary practitioner would not have had a reasonable expectation of success.

The secondary reference of Brashears *et al.* reports on their results from *in vitro* tests that suggest lactic acid bacteria might be a good candidate for a competitive exclusion product to inhibit or eliminate *E. coli* O157:H7. The authors indicate future plans to use the product in cattle-feeding trials but, as of the article's publication date, they had not yet tried the product in live animals. Clearly, the limited *in vitro* tests do not demonstrate what would happen under natural or field conditions. Moreover, there is no teaching to motivate the ordinary practitioner to combine the probiotics of Brashears *et al.* with a vaccine containing the inactivated or killed whole or subunit *E. coli* O157:H7 and provide a reasonable expectation of success in reducing the shedding of *E. coli*. As a probiotic, *Lactobacillus acidophilus* might interfere with the immunogenic activity of the bacterial vaccine. The properties of the combination simply cannot be foreseen from the cited art.

Besides, neither Finlay *et al.* nor Brashears *et al.* teach or imply the use of the inactivated or killed *E. coli* O157:H7 bacterin. The combined references totally fail to teach or suggest all claim limitations. It is certain that the ordinary practitioner would not arrive at the present invention without inventive effort.

In view of the foregoing evidence that the *prima facie* case of obviousness is not established, it is respectfully asked that the rejection of Claim 21 under 35 U.S.C. § 103(a) be withdrawn.

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Accordingly, it is believed that this application is now in condition for an allowance. Favorable treatment is respectfully urged.

Respectfully submitted,

WYETH

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Investigation of On-Farm Management Practices as Pre-Harvest Beef Microbiological Interventions Project Summary

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Study Completed June 2003

Prepared on behalf of the Cattlemen's Beef Board by the National Cattlemen's Beef Association Center for Research & Knowledge Management

Funded by America's Beef Producers

Investigation of On-Farm Management Practices as Pre-Harvest Beef Microbiological Interventions
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Background

Highly publicized outbreaks of food-borne illness since 1993, primarily caused by bacteria such as *E. coli* O157:H7, *Salmonella spp.* and *Listeria monocytogenes*, elicited intense consumer concern about meat safety. In response, regulatory authorities, researchers and the beef industry initiated efforts to implement food safety management systems that would improve microbiological quality. The USDA Food Safety and Inspection Service (FSIS) began initiating new regulatory requirements during the mid-1990s. Packers were required to knife-trim carcasses to remove all visible contaminants, comply with written sanitation standard operating procedures (SSOP), implement Hazard Analysis Critical Control Point (HACCP) systems, and meet microbiological performance criteria and standards for *E. coli* and *Salmonella* as a means to verify HACCP effectiveness and pathogen reduction.

Researchers and beef packers/processors have addressed consumer food safety concerns by developing a variety of methods that are now implemented, or are being further developed, to reduce numbers of bacteria on beef and beef products and improve microbiological safety. These microbiological decontamination technologies include:

- Animal cleaning;
- Chemical dehairing at slaughter;
- Spot-cleaning of carcasses by knife-trimming or steam/hot water vacuuming; and
- Spraying/washing/rinsing of carcasses before evisceration and/or before chilling, with water, chemical solutions and/or steam or hot water.

Enhanced sensitivity tests have shown that the prevalence of *E. coli* 0157:H7 in live cattle and the environment is much higher in fecal and carcass samples than researchers had originally thought. The main objective of this study was to identify effective mitigation strategies that can be used by producers to reduce the carriage and shedding of *E. coli* O157 in market-ready feedlot cattle.

Methodology

Dr. Keith Belk, Colorado State University researcher, tested:

1. *Lactobacillus acidophilus* probiotic (Bovamine Culture Complex Probiotic, Nutrition Physiology Corp.);
2. Neomycin sulfate (NEOMIX®, Pharmacia & Upjohn Company); and
3. An *E. coli* O157:H7 bacterin (FDAH Vaccine, Fort Dodge Animal Health).

Seven variations on the treatments were studied in addition to the control group – the three interventions mentioned above were tested singularly and four treatments applied the intervention strategies in combination. The study was conducted in a commercial feedlot located in Eastern Colorado from March 1, 2003 through May 26, 2003. Researchers collected 1,172 fecal and hide samples from 24 pens of cattle with approximately 200 head of (925 lb) cattle per pen.

Findings
The table below shows the effects of each treatment on *E. coli* 0157 hide and fecal prevalence.

Percent prevalence (and parenthetically, percent difference from control) of positive *E. coli* O157 isolates from hide or fecal samples collected from cattle exposed to one of eight treatments.

Control or Treatment	Percent Positive *E. coli* O157 isolates	
	Hide	Fecal
Control	40.3	45.8
Lactobacillus acidophilus (Bov)	22.7 (43.7)	13.3 (71.0)
Neomycin sulfate (Neo)	8.5 (78.9)	0.0 (100)
E. coli O157:H7 bacterin (Vac)	20.0 (50.4)	14.7 (67.9)
Vac + Bov	16.4 (59.3)	32.9 (28.2)
Vac + Neo	6.7 (83.4)	26.7 (41.7)
Neo + Bov	7.1 (82.4)	1.3 (97.2)
Vac + Bov + Neo	6.7 (83.4)	2.7 (94.1)

Fecal Samples: In the cattle control group, 45.8 percent of the fecal samples tested positive for *E. coli* O157. After treatment with neomycin sulfate, no fecal samples tested positive for *E. coli* O157. The *Lactobacillus acidophilus* and the bacterin vaccine, when administered singularly, resulted in only 13.3 and 14.7 percent of positive tests, respectively. Only 1.3 percent of fecal samples tested positive for *E. coli* O157 after a combined treatment of neomycin sulfate and *Lactobacillus acidophilus*.

Hide Samples: In the cattle control group, 40.3 percent of hide samples tested positive for *E. coli* O157. After treatment with all three interventions (*Lactobacillus acidophilus* probiotic, neomycin sulfate and *E. coli* O157:H7 bacterin), only 6.7 percent of the hide samples tested positive for *E. coli* O157. The same percent (6.7) of samples tested positive following a combined treatment of neomycin sulfate and *E. coli* O157:H7 bacterin as well. When treated with neomycin sulfate and *Lactobacillus acidophilus* in combination, just 7.1 percent of the hide samples tested positive for *E. coli* O157.

Treatment and Status of Interventions: *Lactobacillus acidophilus* probiotic (Bovamine) was fed for 90 days prior to slaughter. This product can be implemented immediately as an intervention strategy.

Neomycin sulfate (NEOMIX® AG 325 Medicated Premix) was fed, according to label, in Type C medicated solid feed at slightly below the recommended dosage. It was fed for three days, and then removed from the ration at least 24 hours before harvest to meet withdrawl requirements. Neomix is currently approved for treatment and control of colibacillosis (bacterial enteritis). The Food and Drug Administration needs to give direction as to whether control of *E. coli* O157:H7 would be considered an "extralabel drug use" under a valid veterinarian/client/patient relationship.

E. coli O157:H7 bacterin was administered two times during the study, 30 days apart. Since the vaccine is still in the experimental stage, U.S. Department of Agriculture-Food Safety and Inspection Service granted slaughter permits for the cattle vaccinated in this study. The checkoff-funded research builds the case for a possible vaccine solution using *E. coli* O157:H7 bacterin.

Implications

The Centers for Disease Control (CDC) estimates that there are 76 million cases of food-borne illness United States annually, with 14 million cases attributed to known pathogens. *E. coli* alone is estimated to account for 76,000 cases of food-borne illness and 76 deaths annually. Multiple intervention strategies to inhibit or eliminate *E. coli* in the beef production process are extremely important to the industry. The results of this study show that three *E. coli* O157 interventions (a microbial feed additive, antimicrobial feed additive and vaccine) produced reductions in *E. coli* O157 prevalence on hides and in fecal materials. There was also an additive effect of combining multiple treatments; the usage of all three interventions produced some of the largest reductions in *E. coli* O157 prevalence on both hides and in fecal samples.